Dice, James F.

BACKGROUND AND FORMAT OF THE CONFERENCE

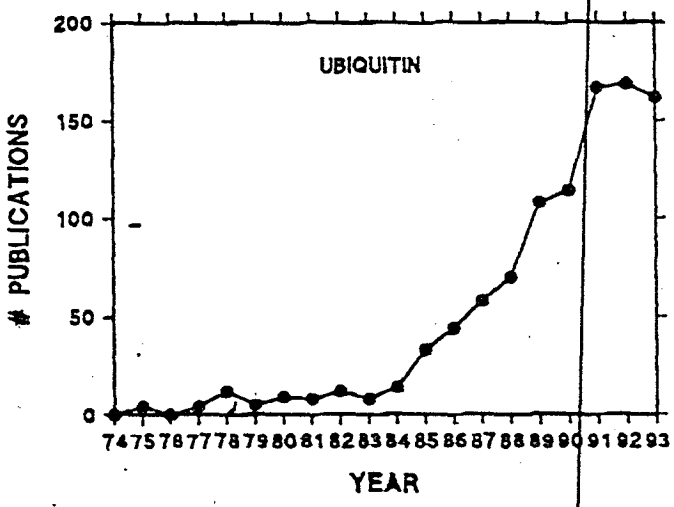
The Federation of American Societies of Experimental Biology (FASEB) facilitates and partially supports a series of weekly summer conferences. In 1989 FASEB sponsored the first meeting on "Ubiquitin and Intracellular Protein Degradation", and this meeting was highly successful and was attended by 98 scientists. The participants unanimously voted to reconvene the meeting on a biennial basis. The 1991 and 1993 meetings were also very successful and were attended by increasing numbers of scientists.

FASEB CONFERENCES ON UBIQUITIN AND PROTEIN DEGRADATION

YEAR	CHAIRS	APPLICATIONS
1989	M. J. Schlessinger A. L. Goldberg	98
1991	A. L. Goldberg R. D. Vierstra	152
1993	R. D. Vierstra J. F. Dice	178

This conference represents the only regularly scheduled meeting devoted to pathways of intracellular protein degradation with emphasis on ubiquitin-dependent proteolysis.

The importance of the conference is reflected in the increasing numbers of publications per year concerning ubiquitin and ubiquitinylation.



The FASEB summer conferences follow the format of the Gordon Research Conferences in that presentation of unpublished material is encouraged, no publication of the proceedings are allowed, and all participants are expected to spend the full 5 days at the conference. The meetings are divided into 9 sessions held in the mornings and in the evenings with the afternoons left open. The Thursday presentations are in the morning and afternoon with an evening banquet and social gathering.

The program of the conference will be advertised several months in advance in Cell, Science, Federation Proceedings, and the FASEB Newsletter. Applicants will be selected by the Chairperson and Vice-Chairperson based on: 1) their scientific accomplishments and speaking abilities, to ensure an outstanding program; 2) their experimental approach, to foster interactions between different disciplines; 3) their geographic and institutional distribution, to ensure that every major research program world-wide is represented; and 4) their age and gender, to ensure representation by younger scientists and women.

Submission of posters will be encouraged, and, based on results from the two previous meetings, at least two poster sessions are planned. A booklet of the poster abstracts will be available at the beginning of the conference, and all conference participants will be encouraged to attend the poster sessions. Approximately 8 posters will be selected for oral presentation in each of the final two sessions of the conference. This selection will be made by the Chair and Co-chair based on submitted abstracts. Special consideration will be given to new people in this field of research and to younger scientists.

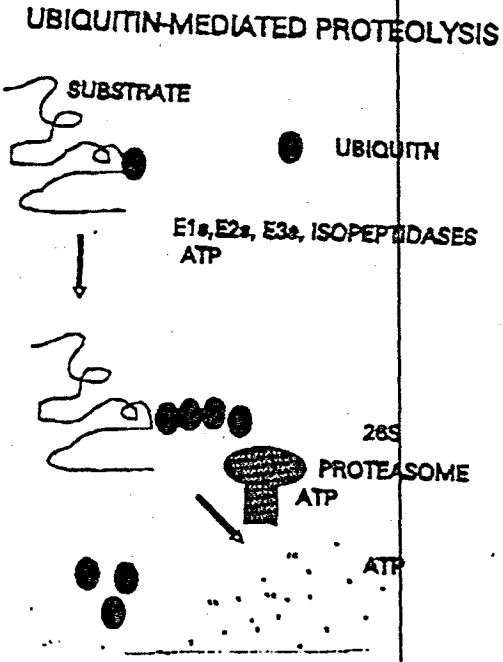
Housing and dining facilities will be provided by the Conference Center at Vermont Academy, Saxton River, VT. The fee for the conference, which includes registration, lodging, and meals, will be approximately $395 per person. Logistical support will be provided by the FASEB staff.

The improvements planned for this meeting compared to the three prior meetings include: 1) An introductory lecture about yeast molecular biology planned for Monday afternoon; 2) Printing and distribution of abstracts of the poster presentations; 3) Having chairs for each session who do not speak in that session; and 4) Increasing the sessions for oral presentations of posters from one to two. In addition, the selection will be made prior to the meeting so that the oral presenters will be prepared and no campaigning during the meeting will influence the decision.

SCIENTIFIC CONTENT OF THE CONFERENCE

Protein degradation is as important as protein synthesis in determining levels of particular enzymes as well as the total protein content within cells. There are multiple pathways of intracellular proteolysis, and rapid progress is being made in

understanding how proteins are targeted for different proteolytic pathways.



Ubiquitin-Dependent Proteolysis

A major breakthrough in understanding a cytosolic pathway of proteolysis was the discovery 14 years ago of the 76-amino acid protein called ubiquitin. Ubiquitin, as its name implies, is an abundant protein in all eukaryotes. It was originally thought to be a mitogenic factor, but was later discovered to be an essential component of a complex cytosolic proteolytic pathway. Ubiquitin is ligated through its C-terminus to an ϵ-amino group of lysine in an acceptor protein. Ligation is catalyzed by 3 different classes of enzymes (E_1, E_2, and E_3). There are many different isozymes of E_2s and E_3s that show some substrate specificity and different intracellular locations. Multiple ubiquitins (ubiquitin chains) target the protein substrate for hydrolysis by a large molecular weight (1,500 kDa) 26S proteasome. The ubiquitins may be cleaved from the substrate protein by isopeptidases associated with the 26S proteasome and then reused for additional rounds of ubiquitination.

There is growing evidence that the 26S proteasome activities are highly regulated. There are intracellular activators and inhibitors of the 26S proteasome, and certain subunits are subject to reversible phosphorylation.

Ubiquitin-dependent proteolysis is the mechanism of degradation of many kinds of abnormal proteins such as those created with biosynthetic errors or by stress-induced denaturation. Ubiquitin-dependent proteolysis is also responsible for the degradation of many short-lived normal proteins including cyclins, phytochrome, p53, c-mos, c-fos, and c-jun.

Ubiquitin genes encode head-to-tail polyubiquitin repeats of up to 52 ubiquitins or a single ubiquitin fused in frame with coding sequences for particular ribosomal proteins. Soon after translation the polyubiquitin or ubiquitin-protein fusions are rapidly processed by a ubiquitin hydrolase.

It is becoming increasingly clear that ubiquitin-tagging plays a role in important cellular processes other the

proteolysis. A single ubiquitin linked to a protein does not cause its rapid proteolysis but may alter subcellular targeting and/or structure of the substrate protein. For example, the incorporation of ribosomal proteins into ribosomes is improved by its expression as a ubiquitin-fusion product. Ubiquitin conjugation has also been implicated in cell cycle progression, chromatin structure, DNA repair, cell surface recognition, signal transduction, cytoskeletal organization, viral assembly, and antigen presentation.

The molecular determinants within proteins that target them for ubiquitin chain conjugation and subsequent degradation are beginning to be understood. One important signal for ubiquitination is the amino terminal amino acid of the protein. Basic and large hydrophobic residues favor ubiquitination, but an acceptor lysine must also be spacially close to the amino terminus and perhaps in a flexible portion of the protein. It is increasingly clear that many proteins are degraded by the ubiquitin-dependent pathway independent of the amino terminal amino acid but dependent upon distinct sequences or structures within the protein.

There is also a ubiquitin cross-reactive protein within cells that is homologous to a ubiquitin dimer. It is also coupled to cellular proteins, but its physiological function is not yet known.

Ubiquitin-Independent Cytosolic Proteolysis

There are probably multiple cytosolic pathways of intracelluler proteolysis that are not dependent on ubiquitin. At least one short-lived protein, ornithine decarboxylase, is degraded by the 26S proteasome but does not require ubiquitin conjugation. The 26 S proteasome can also dissociate to form the 20S proteasome which may be involved in antigen processing. Intracellular activators and inhibitors of the 20s proteasome have been described and are probably important regulators of the 20S proteasome activity.

Calcium-dependent proteases, calpains I and II, also exist in the cytosol as well as associated with the plasma membrane and the cytoskeleton. These proteases are responsible for limited cleavage of certain proteins, but their importance in overall proteolysis remains to be determined.

Proteolytic Pathways within Organelles

Lysosomes:

Lysosomes have long been known to be able to internalize and digest both extracellular and intracellular proteins. Four distinct pathways for internalization of intracellular proteins by lysosomes are now known. The degradation of many plasma

membrane proteins, as well as certain other intracellular membrane proteins, appears to be through vesicle fusion pathways. Lysosomes are also able to internalize cytosolic proteins though microautophagic and macroautophagic processes. An additional pathway of lysosomal proteolysis is selective for cytosolic proteins containing peptide sequences related to KFERQ. The mechanisms by which such proteins are targeted to lysosomes for degradation is similar in many respects to the import of newly synthesized proteins into organelles. For example, heat shock proteins of 70kDa (hsp70s) facilitate the import process.

Recent evidence implicates ubiquitin-conjugation as a necessary process in lysosomal macroautophagy. In temperature-sensitive mutants of E1, autophagic vacuoles form and mature normally up to the degradative step in which autolysosomes are converted to residual bodies. Whether ubiquitination of substrate proteins is required for efficient lysosomal degradation or whether ubiquitination activates some step in the lysosomal proteolytic machinery remains to be established.

Endoplasmic Reticulum:

Selective degradation within the ER was first shown for subunits of the T-cell receptor but has now been shown for a large number of plasma membrane and secreted proteins. This process requires calcium, and potential ER proteases have been identified.

Mitochondria and Chloroplasts:

These organelles contain signal peptidases to process precursor proteins after import, but they also contain other proteolytic pathways presumably responsible for the complete degradation of organellar short-lived proteins. These proteolytic pathways are similar to those described for prokaryotes. For example, a mitochondrial protease has been described that is homologous to the lon protease in E. coli, and the a chloroplast protease has been described that is homologous to the Clp A/P protease from E. coli (see below).

Proteolytic Pathways in Prokaryotes

A detailed understanding of protein degradation pathways in prokaryotes is likely to lead to a better understanding of similar processes in eukayotes. Although E. coli do not contain ubiquitin, they do express an "N-end rule" for protein degradation, and the proteolytic system responsible for this N-end selective degradation is the Clp A/P system. In addition, the lon protease is heat inducible, and this finding lead to experiments showing that the eukaryotic polyubiquitin genes were heat-inducible. Finally, the mechanisms of action of the lon

protease, including its requirement for ATP, are likely to be relevant to eukaryotic ATP-dependent proteases.

Another practical reason for learning more about protein degradation in E. coli is that many cloned proteins of economic and medical interest are rapidly degraded when expressed in bacteria. Expression in strains defective in various proteolytic pathways has already increased yields of several polypeptides.